(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 411 907 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 90308424.2

(51) Int. Cl.5: G01N 21/17, G01N 33/53

(22) Date of filing: 31.07.90

(30) Priority: 01.08.89 US 388193

(43) Date of publication of application:
06.02.91 Bulletin 91/06

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB IT LI LU NL SE

(71) Applicant: ORTHO DIAGNOSTIC SYSTEMS INC.
U.S. Route no. 202
Raritan, New Jersey 08869-0602(US)

(72) Inventor: Schutt, Ernest G.
7 Quail Run
Long Valley, New Jersey 07853(US)
Inventor: Greenwood, David L.
64-D Tea Street
Bound Brook, New Jersey 08805(US)
Inventor: Utberg, Karin
23 Thruway Drive
Bridgewater, New Jersey 08807(US)

(74) Representative: Mercer, Christopher Paul et al
Carpmaels & Ransford 43, Bloomsbury Squareare
London WC1A 2RA(GB)

(54) Scattered total internal reflectance apparatus.

(57) A new immunoassay system is provided for the detection of ligands or ligand binding partners in solution in a heterogeneous format. The invention relies upon the detection of back scattered light from an evanescent wave disturbed by the presence of a colloidal gold label brought to the interface by an immunological reaction. The evanescent wave existing at the interface in turn is the result of a totally internally reflected incident light wave. Placement of the detector at a back angle above the critical angle insures a superior signal-to-noise ratio. Apparatus and methods for scanning, detecting and manipulating light including a scattered total internal reflectance immunoassay system are provided.

IDS
OK

EP 0 411 907 A2

SCATTERED TOTAL INTERNAL REFLECTANCE APPARATUS

This invention relates to apparatus and methods for scanning, detecting and manipulating light including a scattered total internal reflectance immunoassay system.

Many human disease states are identified on the basis of immunoassay techniques which rely upon the specificity between immunoglobulins, whether monoclonal or polyclonal, and their respective binding partners, which may be haptens, antigens, or other analytes, all of which may hereafter be collectively and interchangeably referred to herein as "ligands" and "ligand binding partners." Futhermore, "ligand" also means any molecule having an affinity to bind or complex with a "ligand binding partner", including chalators, immunobinders, nucleic acid strands, bioreceptors, and hydrophobic binders. Over the past fifteen or so years, there has been a substantial amount of effort involved in the development of immunoassay techniques utilizing the so-called sandwich and competitive techniques. The sandwich technique involves the immobilization of an antigen by one antibody and then subsequent labeling of attachment of a second antibody having associated therewith a detectable label.

Reverse immunoassays for the detection of antibody are similar but instead put antigen on the surface for reaction with the sample antibody. Competitive techniques are useful for antigens having only a single epitopic site for reaction with an antibody. Accordingly, and as the name implies, such techniques rely upon the competition of the antigen with another labeled antigen for a binding site on an immobilized antibody. The substitutions necessary for antibody detection tests are obvious and need not be covered here in any great detail.

Of great importance in the laboratory is the development of highly sensitive techniques which can be run in either batch random access, panel, or stat modes. Preferably, such techniques will be homogeneous in nature, i.e., and as used herein, they will be conducted solely within one container without any accompanying requirement to physically separate out components following reactions during the assay.

It is one object of the present invention to provide a new immunoassay system which is highly sensitive and which is homogeneous in nature.

U.S. Patent 3,939,350 to Kronick and the Kronick citations therein referenced describe an immunoassay system which allows for the measurement of biochemical analytes by fluorescence in a liquid sample. Kronick employs a physical phenomenon known under the name of total internal reflectance. This optical phenomenon occurs wherein light, when directed through a high refractive index material toward the interface of that material with a second material having a lower refractive index at greater than a critical angle, all light is reflected from that interface save for a microscopic evanescent wave which propagates into the second material for only a short distance. The second material may, for instance, be water or another aqueous medium in which an assay is being conducted. Kronick noted that when he brought materials which had been fluorescently labeled down to the interface and within the field of the evanescent wave, he could energize the fluorescent molecules and detect fluorescence which then emanated into the overlying solution. The Kronick system, however, looks at fluorescence which cannot be readily modified by alteration of the fluorescent labels in order to suit the system under study. Due to the nature of the specificity of the fluorescent label with respect to the wavelength of the excitation frequency, one is limited to a discrete light source providing the critical excitation frequency. To date, most investigators favor the He-Ne laser light source due to its reliability and low cost as well as the low cost of associated optics. Such a light source, however, brings concomitant difficulties in tailoring fluorescent molecules to be excited by the He-Ne laser output. The organic, inorganic, and bio-organic techniques required are especially difficult to control in the immunoassay arena. Further, Kronick's reliance on fluorescence is accompanied by additional disadvantages associated with bleaching of the fluorescent molecules and generally critical matching of fluorescent molecule excitation wavelength with laser output wavelength necessary to obtain good quantum efficiency.

It is an object of the present invention to provide a new immunoassay system which avoids the disadvantages associated with fluorescent labels and the criticality associated with matching an excitation source.

it is another object of the present invention to employ the principles of total internal reflection but with far greater flexibility regarding the choice of illumination sources.

U.S. Patent 4,181,441 to Noller describes a system similar to that of Kronick. Noller, however, taught that the assay should be conducted by measurement of light absorption in a liquid sample which could then be correlated to the presence of biochemical analytes. Although the Noller system employs different physical principles than the Kronick system, light absorption measurements are similarly subject to poorer signal-to-noise ratios due to small differences in large light signals thereby making such a system inherently

less sensitive than desired.

It is another object of the present invention to avoid employing light absorption measurements while still gaining the advantages to be provided by the total internal reflectance phenomenon.

U.S Patent 4,521,522 to Lundstrom teaches yet another immunoassay based upon reflectance and the use of Brewster's angle. This system relies upon a different optical phenomenon wherein directing a light beam, polarized in the place of incidence, upon an interface, for example that formed between plastic and liquid, results in the transmission of a strong light beam into the liquid when such light strikes the interface at the Brewster angle. At the Brewster angle, substantially no light is reflected.

The Brewster angle is a function of the refractive indices of the two materials as well as the direction of polarization. Lundstrom noted that upon the growth of a biochemical layer at the interface, the Brewster angle condition would be disrupted resulting in increasing light reflectance, particularly at angles less than the Brewster angle. Unfortunately, the Lundstrom assay only works effectively with a wash step since the transmission of the beam into the liquid also results in the generation of light scatter and thus a spurious signal.

It is another object of the present invention to utilize light scatter but to avoid light scatter generated by the transmission of light into the liquid which occurs naturally when light is directed at an interface at the Brewster angle. Accordingly, it is yet another object of the present invention to avoid employing a Brewster angle condition.

It is another object of the present invention to provide an apparatus and method for scanning, detecting and manipulating light.

In accordance with various aspects and the principles of the present invention, there is provided an immunoassay system which utilizes scattered total internal reflectance (STIR) as a measure of the presence of particular ligands to be determined in an aqueous solution. The invention relies in part upon the identification of the critical angle associated with total internal reflectance. The angle is largely a function of the refractive index of the material through which an incident light wave is directed, e.g. plastic, and the relatively lower refractive index of the material in which the immunoassay is being conducted e.g. an aqueous solution. It is measured from a line perpendicular to the interface between the two materials, and thus at its maximum, 90°, will lie in the plane of the interface.

Light directed through the plastic toward the interface formed by the aqueous sample and plastic materials at the critical angle will result in total internal reflectance of the light within the plastic. It is recognized that no materials in the real world are perfect and accordingly, it is preferred that the incident light be directed toward the interface at an angle several degrees greater than the critical angle, most preferably in the range of approximately 6° greater in order to ensure that the basic conditions of total internal reflectance are met. At such an angle, the incident collimated light, preferably from a laser, is totally internally reflected within the plastic save for the propagation of the evanescent wave parallel to the surface of the plastic and approximately 1/4λ from the surface. Similarly, smooth surfaces at the interface are preferred for optimum signal quality. Unlike conventional fluorescent techniques including those of Kronick, the present assay system is flexible with respect to light wavelength since particle size may be readily adjusted to match the available light source (or vice versa) to provide acceptable light scatter. Fluorescent molecules are not readily adjustable with respect to excitation wavelength.

Most ideally, the light source will be a He-Ne light source, however, other lasers with different wavelength outputs have been used and still other sources suggest themselves including light emitting diodes and other nonlaser light sources.

Applicants' immunoassay system further relies upon conventional immunoassay techniques. However, applicants' immunoassay system also employs a particulate label having a higher refractive index than that of the solution, and most preferably also higher than the first light transmissive material, e.g. plastic in the foregoing example. Such particles would include, for instance, red blood cells, other materials having a highly reflective surface such as metallic particles, and nonmetallic substances such as glass or plastics, e.g. latex particles, and the like. Most preferably, colloidal gold is used as a label for the solution phase immunologically active component. While the use of colloidal gold as a label is known, see for example U.S. Patent No. 4,313,734 Leuvering, almost no nonagglutination related uses of the label have been made to date due to the difficulties associated with its detection, particularly in homogeneous type systems. It was surprisingly discovered by the inventors hereof that the unique combination of STIR with colloidal gold has resulted in an extremely efficient and sensitive homogeneous assay system. It is believed, but not known for certain that this is due primarily to the interaction of the colloidal gold particles with the evanescent wave. Indeed, experience implies that particles having an increasingly higher index of refraction than that of the underlying solid generally increasingly scatter light. While particles with indices of refraction less than the underlying solid, providing they are also not equal to that of the aqueous medium, would also scatter

light, such are less preferred.

Assuming for the moment a conventional sandwich technique, one immunoglobulin or ligand binding partner is immobilized on the surface and binds antigen or other ligand to be determined. Thereafter, (or simultaneously, or if not previously) a second immunoglobulin, directed at a second epitopic site on the ligand, and labeled directly or indirectly with colloidal gold, binds to the ligand creating the so-called "sandwich". In this arrangement, the presence of the colloidal gold disrupts the propagation of the evanescent wave resulting in scattered light which may be detected by a photomultiplier or other light sensor to provide a responsive signal. Another important aspect of the present invention involves the physical location of the detector. The detector is ideally placed at an angle greater than the critical angle and in a location whereby only light scattered backward toward the light source is detected. This location thereby ideally avoids the detection of spurious scattered light within the bulk liquid medium.

Another feature of the instant invention is that the immunoassays are diffusion rate controlled and not particularly temperature dependent. This is in strong contrast to ELISA and various other immunoassay techniques wherein temperature control is critical since small changes in temperature in such systems results in wide variations in assay results per unit of time.

It was surprisingly found by the inventors hereof, that as a result of the combination of these elements, rapid, sensitive results could be obtained in a homogeneous environment without requiring the complicated equipment previously associated with colloidal gold assay techniques.

In addition, these elements led to the invention of apparatus to scan, detect and manipulate light in unexpected ways.

In the accompanying drawings:

Fig. 1 depicts an optically transparent member 53 with refractive index n_2 having a fluid contacting surface 52 in contact with fluid 54 having a refractive index n_1 which is less than n_2. The total internal reflectance critical angle 55 measured from a line 50 perpendicular to the plane of the fluid contacting surface 52 is the minimum angle of illumination required to provide total internal reflectance at surface 52. This angle is defined by the equation

$$\theta_C = \sin^{-1}\left(\frac{n_1}{n_2}\right)$$

The critical angle, θc, is only defined in the higher refractive index medium and can range from 0° to 90°. Light propagating from a point on the fluid contacting surface 58 at the total internal reflectance critical angle 55 would follow the path depicted as 57. All light propagating through the optically transparent member 53 from a point on the fluid contacting surface 58 between the plane of the sample fluid contacting surface 51 and the total internal reflectance critical angle 55 of the fluid contacting surface, will propagate in the range depicted as 56.

Fig. 2 is a simplified elevation view of the cuvette and the rotating optics mechanism used to illuminate and read it.

Fig. 3 is cross-section of a cuvette.

Fig. 4 is a perspective view of a cuvette and a paraboloidal reflector which is the first component of the receiving optics.

Fig. 5 depicts an apparatus with laser illumination above critical angle.

Fig. 6 depicts illumination and detection light paths used when illumination is above the critical angle.

Fig. 7 shows data obtained with the apparatus of Figure 5.

Fig. 8 depicts an apparatus with light emitting diode illumination above the critical angle.

Fig. 9 shows data obtained with the apparatus of Figure 8.

Fig. 10 shows data obtained with the apparatus of Figure 2.

Fig. 11 shows data obtained with the apparatus of Figure 2.

The present invention provides an apparatus for detecting the presence of an analyte of interest in a sample. This apparatus comprises a light source; housing means for receiving an optically transparent member having a sample contacting surface, said member in said housing means being disposed such that the sample contacting surface is illuminated with light emitted from said light source; and photodetection means which excludes the detection of light which propagates in a geometric optical path from the light source, said photodetection means being capable of detecting elastically-scattered light which propagates

through the optically transparent member from the illuminated sample contacting surface between the plane of the sample contacting surface and the total internal reflectance critical angle of the sample contacting surface. Within this application, "photodetection means" is defined as a system for detecting photons having a wavelength equal to the wavelength of the illuminating light, and includes combinations of photon detectors (e.g. photomultiplier tubes), lenses, mirrors, light filters, optical fibers, prisms, apertures, and masks. A geometric optical path is the path that a family of light rays will follow based on first order reflection and refraction of idealized surfaces (imperfection free) and ignoring the effects of surface and bulk material imperfections, diffraction, interference, scatter, and partial reflection at surfaces. Further, within this application, "elastically-scattered light" (also referred to herein as "scatter" and "scattered light") means incident light which has been redirected by an object without changing the wavelength of the light, by means other than doppler shifting, due to the difference in the refractive index of the object and its surrounding medium. Fluorescence, also known as inelastic scatter, is the light emitted by a light absorbing molecule after the molecule has absorbed a photon light. The wavelength of the absorbed light is less than the wavelength of the emitted light. Fluorescent light is always of a wavelength different from the light incident on the light absorbing molecule.

"Critical angle", also referred to herein as "total internal reflectance critical angle", is the angle (less than 90°) measured from the line perpendicular to an interface between materials of different refractive indexes, beyond which total internal reflection can occur, and is defined by the equation

$$\theta_c = \sin^{-1}\left(\frac{n_1}{n_2}\right)$$

wherein n_1 is the lower refractive index and n_2 is the higher refractive index of the two mediums forming the interface. The critical angle can only exist in the higher refractive index medium. Light which illuminates the interface from the lower refractive index material at any angle (0° to 90°) cannot be refracted into the higher refractive index medium at an angle greater than or equal to the critical angle. Total internal reflection occurs exclusively when an interface between materials of different refractive indexes is illuminated from the higher refractive index medium beyond the critical angle, causing all the incident illumination to be reflected at the interface unless it is perturbed by diffraction, scatter, or absorption.

The present invention also provides another apparatus for detecting the presence of an analyte of interest in a sample. This apparatus comprises a light source; housing means for receiving an optically transparent member having a sample contacting surface, said member in said housing means being disposed such that the sample contacting surface is illuminated with light emitted from said light source which propagates through the optically transparent member at angles between the plane of the sample contacting surface and the critical angle for total internal reflectance; and photodetection means which excludes the detection of light which propagates in a geometric optical path from the light source, said photodetection means being capable of detecting elastically-scattered light which propagates through the optically transparent member from the illuminated sample contacting surface between the plane of the sample contacting surface and the total internal reflectance critical angle.

Suitable light sources for the apparatuses of the present invention provide collimated or uncollimated light, polarized or unpolarized light, or monochromatic or polychromatic light. Preferred light sources include lasers (e.g., He-Ne lasers), light emitting diodes (LEDs), flash lamps, arc lamps, incandescent lamps, and fluorescent discharge lamps.

Suitable optically transparent members, e.g., cuvettes, are comprised of glass, quartz, silicon, plastics such as polycarbonate, acrylic, or polystyrene, or oils comprising silicone or high molecular weight hydrocarbons.

Suitable photodetection means comprise photon detectors such as photomultiplier tubes, photodiodes (e.g., PIN diodes and gallium-aluminum-arsenide diodes), cadmium sulfide photoresistive cells, phototubes, and pyrolytic detectors.

Also provided is a method for detecting the presence of a light scattering molecule on the surface of an optically transparent material. This method comprises illuminating said light scattering molecule, detecting light scattered elastically by said light scattering molecule which propagates through said optically transparent material between the plane of the surface of the optically transparent material on which the light scattering molecule is located and the total internal reflectance critical angle of the surface on which the

light scattering molecule is located, and correlating detected, elastically-scattered light to the presence of the light scattering molecule on the surface of the optically transparent material. Within this application, an "evanescent wave" means a nonpropagating light wave such as a wave in the region of a surface on the side of the surface opposite the side of illumination, produced when the illuminating light undergoes total internal reflection. Also within this application a "light scattering molecule" means a molecule which causes incident light to be elastically scattered. "Molecule" includes, in the case of crystalline and elemental materials, two or more atoms.

Still further, the present invention provides a method for detecting the presence of a light scattering molecule on the surface of an optically transparent material. This method comprises illuminating said light scattering molecule with an evanescent wave resulting from a light wave which propagates through said optically transparent material, detecting light scattered elastically by said light scattering molecule which propagates through said optically transparent material between the plane of the surface of the optically transparent material on which the light scattering molecule is located and the total internal reflectance critical angle of the surface on which the light scattering molecule is located, and correlating detected, elastically-scattered light to the presence of the light scattering molecule on the surface of the optically transparent material.

Further provided is a method for detecting an analyte in a fluid sample wherein said analyte is a ligand of a ligand - ligand binding partner pair. This method comprises the steps of:

a) Providing an optically transparent material having a refractive index greater than the refractive index of said fluid sample, said optically transparent material having a sample contacting surface to which a plurality of ligand binding partners of said ligand - ligand binding partner pair are immobilized;

b) further providing light scattering particle-labeled ligands capable of forming complexes with said immobilized ligand binding partners;

c) contacting said fluid sample and said light scattering particle-labeled ligands with said sample contacting surface under conditions such that said analyte and said light scattering particle-labeled ligands each form complexes with said immobilized ligand binding partners;

d) illuminating said complexes with an evanescent wave resulting from a light wave which propagates through said optically transparent material;

e) detecting light scattered elastically by said light scattering particles of said complexes;

f) correlating elastically-scattered light to the presence of complexes on said sample contacting surface; and

g) comparing the presence of complexes on the sample contacting surface with the presence of complexes on a sample contacting surface for a standard control, thereby detecting the analyte in the fluid sample.

Within this application, "particle" means one or more molecules. "Labeled" means directly linked, e.g., conjugated, cross-linked, or adsorbed, or indirectly linked, e.g., linked via an antibody.

Further yet is provided a method for detecting an analyte in a fluid sample wherein said analyte is a ligand of a ligand - ligand binding partner pair. This method comprises the steps of:

a) providing an optically transparent material having a refractive index greater than the refractive index of said fluid sample, said optically transparent material having a sample contacting surface to which a plurality of ligand binding partners of said ligand - ligand binding partner pair are immobilized;

b) further providing light scattering ligands capable of forming complexes with said immobilized ligand binding partners;

c) contacting said fluid sample and said light scattering ligands with said sample contacting surface under conditions such that said analyte and said light scattering ligands each form complexes with said immobilized ligand binding partners;

d) illuminating said complexes;

e) detecting light scattered elastically by light scattering ligands of said complexes and which propagates through said optically transparent material from the sample contacting surface between the plane of the sample contacting surface and the total internal reflectance critical angle of the sample contacting surface;

f) correlating elastically-scattered light to the presence of complexes on said sample contacting surface; and

g) comparing the presence of complexes on the sample contacting surface with the presence of complexes on a sample contacting surface for a standard control, thereby detecting the analyte in the fluid sample.

Within this application, "light scattering ligands" means ligands or light scattering particle labeled-ligands which cause incident light to be elastically scattered.

Further still is provided a method for detecting an analyte in a fluid sample wherein said analyte is a ligand of a ligand - ligand binding partner pair. This method comprises

a) providing an optically transparent material having a refractive index greater than the refractive index of said fluid sample, said optically transparent material having a sample contacting surface to which a plurality of ligand binding partners of said ligand - ligand binding partner pair are immobilized;

b) further providing light scattering ligands capable of forming complexes with said immobilized ligand binding partners;

c) contacting said fluid sample and said light scattering ligands with said sample contacting surface under conditions such that said analyte and said light scattering ligands each form complexes with said immobilized ligand binding partners;

d) illuminating said complexes with an evanescent wave resulting from a light wave which propagates through said optically transparent material;

e) detecting light scattered elastically by light scattering ligands of said complexes and which propagates through said optically transparent material from the sample contacting surface between the plane of the sample contacting surface and the total internal reflectance critical angle of the sample contacting surface;

f) correlating elastically-scattered light to the presence of complexes on said sample contacting surface; and

g) comparing the presence of complexes on the sample contacting surface with the presence of complexes on a sample contacting surface for a standard control, thereby detecting the analyte in the fluid sample.

In one embodiment of the invention, the methods provided herein may be performed wherein the sample contacting surface is contacted with the fluid sample before being contacted with the ligands. In another embodiment of the invention, the sample contacting surface is contacted with the ligands before being contacted with the fluid sample. In yet a further embodiment of the invention, the sample contacting surface is simultaneously contacted with said fluid sample and the ligands. In still another embodiment of the invention, the sample is mixed with the ligands so as to form a mixture, and the mixture is contacted with the sample contacting surface.

Furthermore, in a preferred embodiment of the invention, light scattering particle-labeled ligands comprise ligands labeled with colloidal gold particles.

In still another embodiment of the invention, a method is provided for detecting an analyte in a fluid sample. In this method the analyte is a ligand having an epitope for which a first ligand binding partner is specific and an epitope for which a second ligand binding partner is specific. The method comprises:

a) providing an optically transparent material having a refractive index greater than the refractive index of said fluid sample, said optically transparent material having a sample contacting surface to which a plurality of first ligand binding partners are immobilized;

b) further providing light scattering particle-labeled second ligand binding partners;

c) contacting said fluid sample and said light scattering particle-labeled second ligand binding partners with said sample contacting surface under conditions such that immobilized first ligand binding partner : analyte : light scattering particle-labeled second ligand binding partner complexes are formed;

d) illuminating said complexes with an evanescent wave resulting from a light wave which propagates through said optically transparent material;

e) detecting light scattered elastically by said light scattering particles of said complexes;

f) correlating elastically-scattered light to the presence of complexes on said sample contacting surface; and

g) comparing the presence of complexes on the sample contacting surface with the presence of complexes on a sample contacting surface for a standard control, thereby detecting the analyte in the fluid sample.

Still another method is provided for detecting an analyte in a fluid sample, wherein said analyte is a ligand having an epitope for which a first ligand binding partner is specific and an epitope for which a second ligand binding partner is specific. This method comprises:

a) providing an optically transparent material having a refractive index greater than the refractive index of said fluid sample, said optically transparent material having a sample contacting surface to which a plurality of first ligand binding partners are immobilized;

b) further providing light scattering second ligand binding partners;

c) contacting said fluid sample and said light scattering second ligand binding partners with said sample contacting surface under conditions such that immobilized first ligand binding partner : analyte : light scattering second ligand binding partner complexes are formed;

d) illuminating said complexes;

e) detecting light scattered elastically by said light scattering second ligand binding partners of said complexes and which propagates through said optically transparent material from the sample contacting surface between the plane of the sample contacting surface and the total internal reflectance critical angle of the sample contacting surface;

f) correlating elastically-scattered light to the presence of complexes on said sample contacting surface; and

g) comparing the presence of complexes on the sample contacting surface with the presence of complexes on a sample contacting surface for a standard control, thereby detecting the analyte in the fluid sample.

Within this application, "light scattering second ligand binding partners" means second ligand binding partners or particle labeled second ligand binding partners which cause incident light to be elastically scattered.

Still further is provided a method for detecting an analyte in a fluid sample wherein said analyte is a ligand having an epitope for which a first ligand binding partner is specific and an epitope for which a second ligand binding partner is specific. This method comprises:

a) providing an optically transparent material having a refractive index greater than the refractive index of said fluid sample, said optically transparent material having a sample contacting surface to which a plurality of first ligand binding partners are immobilized;

b) further providing light scattering second ligand binding partners;

c) contacting said fluid sample and said light scattering second ligand binding partners with said sample contacting surface under conditions such that immobilized first ligand binding partner : analyte: light scattering second ligand binding partner complexes are formed;

d) illuminating said complexes with an evanescent wave resulting from a light wave which propagates through said optically transparent material;

e) detecting light scattered elastically by said light scattering second ligand binding partners of said complexes and which propagates through said optically transparent material from the sample contacting surface between the plane of the sample contacting surface and the total internal reflectance critical angle of the sample contacting surface;

f) correlating elastically-scattered light to the presence of complexes on said sample contacting surface; and

g) comparing the presence of complexes on the sample contacting surface with the presence of complexes on a sample contacting surface for a standard control, thereby detecting the analyte in the fluid sample.

In one embodiment of the present invention, the methods provided herein may be performed wherein the sample contacting surface is contacted with the fluid sample before being contacted with the light scattering particle-labeled second ligand binding partners or the light scattering second ligand binding partners. In another embodiment of the invention, the methods described herein may be performed wherein the sample contacting surface is contacted with the light scattering particle-labeled second ligand binding partners or the light scattering second ligand binding partners before being contacted with said fluid sample. Still further, the sample contacting surface may be simultaneously contacted with the fluid sample and the light scattering particle-labeled second ligand binding partners or the light scattering second ligand binding partner, e.g., the fluid sample may be mixed with the light scattering particle -labeled second ligand binding partners or the light scattering second ligand binding partners so as to form a mixture, and the mixture is contacted with the sample contacting surface.

In a preferred embodiment of the invention, the light scattering particle-labeled second ligand binding partners comprise second ligand binding partners which are labeled with colloidal gold particles.

Finally, an apparatus is provided for detecting the scattered internal reflectance as a measure of particular ligands to be determined in an aqueous solution.

Further provided is an apparatus for scanning a plane polarized light source comprising first polarization means for converting plane polarized light to circularly polarized light, means for detecting said circularly polarized light received from said first polarization means and second polarization means for reconverting said directed circularly polarized light into plane polarized light so that the plane of polarization of said light will be dependent only upon the orientation of said means for reconverting said circularly polarized light into plane polarized light.

Further provided is a method for scanning a plane polarized light source comprising converting plane polarized light to circularly polarized light, directing said circularly converted light and reconverting said directed circularly polarized light into plane polarized light so that the plane of polarization of said light will

be dependent only upon the orientation of said means for reconverting said circularly polarized light into plane polarized light.

Further provided is an apparatus for detecting light comprising first paraboloidal reflecting means and second paraboloidal reflecting means whose axis is nearly coincident with and nearly symmetrical to said first reflecting means.

Further provided is a method for detecting light comprising reflecting light from first paraboloidal reflecting means to second paraboloidal reflecting means whose axis is nearly coincident with and nearly symmetrical to said first reflecting means.

Still further, an apparatus for detecting light is provided comprising first paraboloidal reflecting means and second ellipsoidal reflecting means having its axis nearly coincident with and nearly symmetrical to said first paraboloidal reflecting means and its vertex to nearest focus distance substantially equal to the vertex to focus distance of said first paraboloidal reflecting means.

Further provided is a method for detecting light comprising reflecting light from a first ellipsoidal reflecting means to a second paraboloidal reflecting means.

Further provided is an apparatus for dissipating the specular reflection of light occuring when substantially collimated light leaves a material with a refractive index greater than its surroundings comprising two planar surfaces of said material aligned relative to each other so that said substantially collimated light is multiply specularly reflected until said collimated light is substantially dissipated from said material.

Further provided is a method for dissipating specular reflection of light occuring when substantially collimated light leaves a material with a refractive index greater than its surroundings comprising aligning two planar surfaces within 10 degrees of parallel so that said substantially collimated light is multiply specularly reflected until said collimated light is substantially dissipated.

First Series of Experiments

An apparatus embodying the principles of STIR was constructed utilizing an equilateral flint glass prism, Model 01-PES-007, obtained from Melles-Griot. The prism was mounted on a support with one side held horizontal. An antibody-coated cuvette in the form of a microtiter well, available from Dynatech under the trade name IMMULON TWO (styrene) was optically coupled to the horizontal prism surface with standard microscope oil. A five milliwatt helium neon laser (Hughes 3225-H-PC) was used to illuminate part of the cuvette's bottom surface at an angle 6° past the critical angle. Optionally, a cylindrical lens may be used to assist in focusing the laser light beam.

The critical angle was first determined by filling an uncoated cuvette, optically mounted on the prism, with a scattering aqueous medium comprising a mixture of colloidal gold sol produced pursuant to the method reported in Scanning Electron Microscopy Vol II, 9-31 (1981), Sear Inc., AMF, O'Hare, Chicago, generating particle sizes of about 30 to 50 nm and serum. The prism was rotated along an axis transverse to the axis of the incident light until the laser beam path, visible inside the cuvette, optically disappeared indicating that substantially all of the incident light was being reflected at the cuvette-liquid interface, the internal reflectance phenomenon known to occur at the critical angle. This critical angle between a perpendicular line through the surface having the optically mounted cuvette and the laser beam was measured, the prism was reinstalled to provide a horizontal surface and the laser adjusted to illuminate the surface internally through the prism at an angle equal to 6° plus the critical angle. While a polarized laser was used with its polarization aligned with the electric field parallel to the plane of the styrene liquid interface, such is merely preferred but not necessary. Indeed virtually any collimated illumination source will serve. Similarly, while a prism was convenient, any optical coupling device for directing illumination toward the aqueous solid interface may be used such that total internal reflectance can be achieved by that interface.

A photodetector (Hamamatsu No. G1742 photodiode) was positioned at an angle above the critical angle but less than 90° at a position physically near the laser such that it would detect light scattered back toward the laser. In this position, minimal laser light is detected prior to the assay despite imperfections present at the interface.

Thus, placement of the photodetector above the critical angle is important in order to insure that light propagating through the solution, e.g., stray light or secondary light scatter induced by irrelevant sources, cannot reach the detector. As a related advantage, this greatly reduces the effect of the sample's color or turbidity and of bubbles present at the liquid interface.

The electrical signal from the photodetector was electrically coupled to a high gain current amplifier

(Keithley Electrometer 610-C) and the output recorded on a strip chart recorder or digitally recorded by a computer data acquisition system (HP controller 3497A with HP 9836 computer). Reaction rates were then graphically determined on the recorder chart or calculated conventionally employing the computer.

Example 1 - hCG Sandwich Assay

An anti-hCG antibody-coated cuvette (coated by standard physical adsorption) was positioned on the oil-coated prism with a laser internally reflecting off the center of the cuvette. 35 µls of assay buffer (0.01 M phosphate buffered saline at a pH of 7.4 containing 1% bovine serum albumin, 1 M NaCl and 1.5 mg/ml mouse IgG) was added to the cuvette. 50 µls of nonblocking, anti-hCG antibody coupled with colloidal gold (approximately 44 nm in size) was then added and mixed by pipette aspiration. 25 µls of serum sample or serum-based standard (Gilford) was then added to the cuvette and mixed. The intensity of the scatter signal was recorded by a strip chart recorder and by a digital data acquisition system. The reaction rate was permitted to equilibrate for the first five minutes to permit the system to become linear and then measured kinetically during the next five minutes. Reaction rates (e.g. signal slopes) of unknown serum samples were compared to the reaction rates of standards in order to compute hCG concentrations. The results were as follows:

Std.	Signal Slope (arbitrary units)
0 mIU	1.00
10 mIU	7.43
25 mIU	16.33
50 mIU	32.03
100 mIU	68.67
200 mIU	130.97

Example 2 - Test For Antibody (Reverse hCG Sandwich Assay)

hCG antigen was coated onto Immulon cuvettes and positioned on the oil-coated prism as in Example 1. 50 µls of colloidal gold (approximately 45 nm) coated with hCG was added to the cuvette along with 35 µls of assay buffer as described in Example 1, and mixed. 25 µls of mouse monoclonal anti-hCG containing standard (diluted in pH 8.3 HEPES/TRIS 0.225 M +0.5% BSA) was added and mixed. After a five-minute delay for equilibration, the rate was measured as in Example 1. As anti-hCG concentrations were increased up to 10 µgs per ml, increasing rates of light scatter were observed with rates decreasing above this concentration giving the expected hook effect (e.g. insufficient labeled and immobilized antigen to accomodate all of the antibody present). The data was:

Mouse IgG Conc. (ng)	Signal Slope (arbitrary units)
0 ng	8.02
10 ng	10.27
100 ng	12.35
1 µg	75.84
10 µg	91.39
100 µg	37.00

Example 3 - Competition With Antigen-Coated Cuvette

Thyroxin (T_4) was covalently coupled to BSA with approximately 20 T_4 molecules per BSA molecule employing the following procedure. T_4-BSA conjugate was prepared from coupling BSA with T_4MC, L-Thyroxinyl-4-(N-maleimido-methyl)-cyclohexane-1- carbonate, through a nucleophilic addition at pH 9-10 by amino groups of BSA to the maleimido group of T_4MC. T_4MC was derivatized from SMCC, succimimidyl-4-(N-maleimido-methyl)-cyclohexane-1-carboxylate (Pierce Chemical), with L-Thyroxine by amidation at neutral pH. T_4-BSA conjugate was absorbed to commercial, strip microtiter wells by incubating 0.1 mls of 0.17 mgs per ml of the conjugate in 0.01 M phosphate buffer adjusted to a pH of 7 at room temperature for 18 hours. The wells were washed three times with 0.25 M HEPES/TRIS buffer (containing 0.05% NaN_3, 0.15 M NaCl at a pH of 8.3). The wells were then incubated for 72 hours at room temperature with 0.2 mls of HEPES/TRIS buffer plus 1% BSA. The wells were then again washed three times with HEPES/TRIS buffer and stored with buffer at 4° C. until use.

Colloidal gold having an average diameter of 40 nm was coated with monoclonal anti-T_4 IgG by previously described methods. The strip well cuvettes were mounted on the prism as in Example 1 and 65 µls of pH 7.4 PBS containing 0.02% NaN_3 and 2% bovine gammaglobulin was added to the cuvette followed by 10 µls of T_4 standard in the same buffer. 25 µls of anti-T_4 antibody coated colloidal gold was then added to the cuvette and mixed. The reaction rate was measured after an equilibration period. As expected, increasing T_4 concentrations correlated with decreased signal rates from back scattered light signal as follows:

T_4 (µg/dl)	Signal Slope (arbitrary units)
0	51.1
2	41.9
4	25.3
8	9.08
12	6.51
24	2.96

Example 4 - Competition With Antigen-Coated Colloidal Gold

Immulon strip well cuvettes were coated with 0.1 mls of 5 µgs per ml of anti-digoxin and 0.1 M KPO_4 at a pH 7.4 and stored at 4° C. until use. The wells were then washed three times with 0.01 M PBS at a pH 7.4. Colloidal gold particles having an average diameter of 40 nm were coated with 1 mg per ml of digoxin--BSA conjugate (approximately 5 digoxins per BSA molecule) by the method set forth in an article by T. W. Smith, Biochemistry 9:331-337 (1970) and then diluted 1 to 4. 35 µls of buffer (0.01 M PBS, 1.0 M NaCl, 1% BSA at pH 7.6) was added to the cuvette followed rapidly by the addition of 25 µls of serum samples or serum base standard and 50 µls of digoxin-coated colloidal gold suspension and mixed. The reaction rate was measured during the next five minutes and the results observed. Increasing digoxin concentrations resulted in reduced reaction rates as follows:

Digoxin (ng/ml)	Signal Slope (arbitrary units, 2 runs)
0	372, 296
0.25	127, 86
0.50	30, 29

Example 5 - Internalized Kinetic Calibrator

It will be recognized that there may be variation from well to well between assays as well as between liquid reagents added to the wells. These differences will result in variations in kinetic responses which could, without correction, lead to erroneous results. One preferred method of correction is to utilize an internalized kinetic calibrator. To do so, a low level control sample is added to the well at the beginning of every assay and the rate of reaction monitored for a short time prior to the addition of the sample to the same well. The control sample can thus be used to calibrate each individual well, e.g. measuring the well's sensitivity and using that information to correct the sample readings, thereby obviating differences in structural or reagent coating uniformity. Accordingly, homogeneous rate assays can be ideally performed by first adding a control sample and monitoring the level of detector output. As a related advantage, this procedure will eliminate the need to perform duplicate assays thereby saving in time and resource expenditures. Such a calibration procedure will also obviate the sample to sample variations in light scattering efficiency of the particles which is a strong function of the index of refraction of the individual sample. The following example of the procedure demonstrates the principles involved.

Molded polycarbonate cuvettes were adsorption coated with anti-hCG antibody. 150 µls of assay buffer (from Example 1), 100 µls of anti-hCG coated colloidal gold (approximately 40 nms diameter) and 75 µls of Gilford stripped serum based 10 mIU/ml calibrator were added to each cuvette and mixed. After 5 minutes incubation, the rate of increase of scattered light intensity (slope) was measured during the next 5 minutes. After recording this calibration slope, 75 µls of Gilford serum based standard was added as sample, mixed and incubated 5 minutes before reading the scattered light slope during the next 5 minutes. The net calibrated slope of each cuvette was calculated by the equation:

Net calibrated slope = [slope of standard/slope of calibrator] - 0.8826

Where 0.8826 was the average slope of six zero hCG standards divided by their respective calibration slopes.

The CV (coefficient of variation) of six replicates of the following standards were calculated on the basis of the net calibrated slope and compared to the uncorrected slope of these standards. The data was as follows:

mIU/ml of standard	CV of uncorrected	CV of Net Calibrated slope
10 mIU/ml	18.31%	10.79%
50 mIU/ml	30.3 %	21.42%
100 mIU/ml	18.86%	5.88%
200 mIU/ml	33.63%	30.86%

In all cases, it can be seen that greater accuracy and repeatability was obtained using the internal calibration method.

Example 6 - Competitive hCG Assay Using Latex Particles

Immulon strip wells were coated as stated in Example 1 above. 35 µls of assay buffer was added to each well. 25 µls of hCG dissolved in stripped serum (Gilford) was then added and mixed. After a 5 minute incubation, 50 µls of "Ortho Beta-hCG Slide Test for Pregnancy" hCG coated styrene latex (0.375 micron diameter) (Ortho Diagnostic Systems Inc.) was added and mixed. The reaction rate was permitted to equilibrate for 5 minutes while the slope of the scattered light signal was calculated during the next 5 minutes. The results were as follows:

HCG Standard Concentration	Signal Slope (arbitrary units)	Average
223,875 mIU/ml	3.61, 3.76, 6.04	4.47
22,387	8.96, 9.02, 9.25	9.08
2,238	118, 122, 144	128
223	158, 162, 187	169
22.3	148, 157, 196	167
2.2	138, 142, 161	147

Example 7 - Direct Red Cell Antigen Test using Red Cell Particle (approximately 8 micron diameter)

Polycarbonate cuvettes were coated by adsorption with anti-D (anti-Rh_o) for an RH factor test and with anti-A for an ABO blood group test. 0.5 ml of human whole blood was centrifuged, resuspended in 5 ml of phosphate buffered saline (PBS) pH 7.4, centrifuged and resuspended in 2 ml of PBS. 300 μls of this sample suspension was added to the coated cuvette and mixed. After a 2 minute incubation, the slope of the scattered light intensity was calculated over the next 8 or 18 minutes. The results were as follows:

Slope in anti-A Coated Cuvettes	
Red Cell Phenotype Sample Blood Type (RH Type)	Slope (Time)
A-	267 (8 min)
A+	240 (18 min)
B+	-18.6 (8 min)
O	14.9 (18 min)
Slope in anti-D Coated Cuvettes	
Sample Blood Type (RH Type)	Slope (Time)
A+	56.6 (18 min)
B+	10.2 (18 min)
O-D-(high positive RH)*	32.3 (18 min)
A-	4.3 (18 min)
O-	4.5 (18 min)

*rare blood type

It will be readily recognized by those skilled in the art that a certain amount of physical manipulation may be made to this system without substantially departing from either the spirit or the scope of the present invention. For example, the cuvettes and prism assembly may be one integral unit wherein the cuvette microtiter well is molded with a plastic prism forming part of the cuvette. Similarly, while an angle 6° above the critical angle has been found most preferred, it will be recognized that dependent upon the optical characteristics of the illumination source and the photodetector, certain variations above the critical angle may be more optimal and are to be deemed equivalent to the angle set forth herein. Further, measurements may take place on the side or bottom of the cuvette.

Further, while colloidal particles such as gold, latex and red blood cells have been described in the Examples, it should be recognized that particles and their particular size range are not to be deemed limitations but are merely exemplary of the wide range of possibilities. Indeed, the size of particles generally should be chosen with consideration given to the wavelength of the light in the liquid medium (in turn a function of the refractive index of the medium), the index of refraction of the particle should ideally be chosen with consideration given to the index of refractions of the aqueous medium and the solid so that the net effect is an optimum signal, most advantageously obtained when resonance of the system occurs. While

predictability is exceedingly difficult given the current level of understanding of these complicated interactions, the actual optimization procedures are relatively simple and easily performed by those skilled in the art.

Second Set of Experiments

Referring to Figure 2, a rectangular plate **1** is rotated at constant speed (by a motor, belt, and pulleys, not shown) in a horizontal plane about a vertical axis **2**.

The following components are mounted on the rotating plate and travel with it: two front surface mirrors **5** and **6** (Melles Griot 02MFG000); a 150 mm focal length lens **7** (Melles Griot 01LPX237); a quarter-wave retardation plate **10** (Melles Griot 02WRM013-632.8); a paraboloidal reflector (axial cross-section **13**) with a clearance notch to permit a laser beam to pass through; a second paraboloidal reflector **14** without a notch; and an aperture plate **15** (0.25 mm thick with a 2 mm hole centrally located about the rotational axis). The paraboloidal reflectors **13** and **14** have a focal length of 10.2 mm (Aero Research Associates 484-001). These are off axis segments of parabolic reflectors mounted with the optical axis of their parent parabolas nearly coincident.

Beneath the rotating assembly is a polarized He-Ne laser **18** (5 mW, Melles Griot 05LHP151). The laser beam goes through a first quarter-wave retardation plate **8** (same as **10**), is reflected off a front surface mirror **4** (same as **5** and **6**), and passes through a second quarter-wave retardation plate **9** (same as **10**). Each quarter-wave plate (**8**, **9** and **10**) is mounted so that it can be rotated in a plane perpendicular to the laser beam for adjustment and can be locked in a position when correctly adjusted. When the laser beam emerges from plate **9**, it is coincident with the axis of rotation **2** of the rotating optical assembly.

The optical signal obtained from the scatter at cuvette surface 12 is sensitive to the polarization of the incident light. This signal dependence upon the incident light polarization has been observed in other forms of optical measurement such as fluorescence, raman spectroscopy, plasma clot detection, and reflectivety. If a rotating mirror is used to scan a plane polarized light beam across a series of targets or samples, the plane of the polarized light rotates about the axis of the light beam as this beam of light is scanned. Thus each position of the rotating scanning mirror illuminates each target with a different polarization alignment. It was surprizingly discovered that, a uniform polarization condition can be maintained independent of the scanning mirror position by converting the light incident on the mirror to circularly polarized light before scanning and then reconverting it to plane polarized light after redirection.

The three quarter-wave plates were used in this embodiment because the optical signal obtained from the scatter at cuvette surface **12** is sensitive to the polarization of the incident light. Thus in order to assure uniform results about the circle of cuvettes, the beam must be in the same polarization condition at all positions. To achieve this, a laser **18** producing a plane polarized beam was employed. This beam is given circular polarization by passing it through a properly oriented quarter-wave retardation plate **8**. A second retardation plate **9** is provided to permit fine tuning to compensate for imperfections in the characteristics of the first retardation plate. A third quarter-wave plate 10 on the rotating member is used to produce plane polarized light with the electric field parallel to the plane of cuvette surface **12**. While retardation plates were used in this experiment, magneto-optic devices may also be used to obtain the same effects on polarization.

The lens **7** is used to converge the laser beam **3** from 0.8 mm diameter as it enters the lens to 0.2 mm diameter at the total internal reflection surface **12**. The small diameter facilitates multiple readings, which are averaged to improve instrument precision.

A plate **19** with receptacles for forty cuvettes is mounted above the rotating optics assembly. The receptacles are disposed in a circle whose center is the axis of rotation of the rotating optics assembly. One of the cuvettes is shown at **11**.

The pads 107 and 109 enable precise location of the cuvette in the receptacle. (The surfaces 111 and 113 protect surface 21 from dirt or damage.) As the optical assembly rotates, it presents the laser beam and the receiving optics to each cuvette in turn. At each cuvette a plurality of readings is obtained as the assembly moves past the optical face of the cuvette.

Referring to Figure 3, the laser beam **3** enters face **21**, travels through the transparent plastic material to surface **22**, where it undergoes total internal reflection, and exits the cuvette at surface **24**. However, as the beam exits surface 24 a small percentage of the laser energy is reflected due to the refractive index mismatch of air and plastic. Since this reflected energy is significantly large relative to the signal energy, it is important that it be directed away from the detector. Surface 24 is therefore angled so that the reflection

goes toward surface 101 of the cuvette. Similarly, surface 101 is angled so that the reflection is directed away from the detector, specifically, to a second point on surface 24. The angles of surface 24 and surface 101 are designed so that many reflections take place in the protrusion defined by surfaces 24, 103, and 101: specifically, surfaces 24 and 101 converge slightly as they approach surface 103.

At each reflection, most of the energy escapes the plastic, so that by the time the reflecting beam reaches surface 103, virtually all the energy is gone, and only a negligible amount ever returns to the detector. Thus the protrusion defined by surfaces 24, 103, and 101 constitutes a light trap which protects the integrity of the signal.

A light trap of this configuration was surprisingly found to almost completely remove the unwanted illumination from the cuvette after it had generated the signal. Many forms of spectroscopy, e.g. nephelometry, fluorescence, raman spectroscopy and absorbance spectroscopy, employ an illuminating beam of light. Errors in measurement in these forms of spectroscopy result when the illuminating beam is reflected off the air-cuvette interface while exiting the cuvette and further excite signal in the sample or enter the receiving optics directly. A light trap of this design can eliminate these errors. This light trap design is based on multiple reflections and thus functions over a wide range of wavelengths. While anti-reflective coatings can perform a similar task this light trap functions for a wide range of wavelengths, is less expensive, as it requires no post molding processing and does not involve coatings that can interfere with subsequent protein coating of a disposeable.

A mask 105 blocks any light which is exiting surface 101 from reaching the detector. The analyte solution is contained in the well **20**. Surface 22 is maintained in a vertical orientation so that particles do not settle on it. The signal-generating scatter occurs at **12**.

The laser beam **3** is introduced into the entry face **21** slightly below the normal to the surface, so that any surface reflection of the laser beam is directed away from the receiving optics. The beam is refracted at the entry surface so that it impinges on the surface **22** at an angle from the normal to surface **22** greater than the critical angle. Thus it is totally internally reflected.

The sector (of a complete paraboloid) which is used at **13** (Figures 2 and 4) to collect the optical signal generated at the total internal reflection surface **12** is determined by the requirement to be at an angle greater than the critical angle and whereby only light scattered backward toward the light source is detected. Areas of the paraboloidal reflector **13** at lesser angles are masked with matte black pressure-sensitive paper tape. Thus only light originating from the total internal reflectance critical angle **25** to rays originating parallel to surface **12**, shown as **23** in Figure 2, are accepted.

The paraboloid is located so that the scatter source **12** is at its focus. Rays originating at the focus of a paraboloid are reflected parallel to its axis, so the signal light **23** to **25** (Figures 3 & 4) is transmitted as a beam **17** toward the second paraboloid **14**. (See Figure 4 for detail.)

Rays parallel to the axis of a paraboloid converge at the focus after reflection. The second paraboloid **14** concentrates the signal energy at its focus, where the aperture plate **15** is mounted. The aperture plate prevents stray light (not originating from the scatter source **12**), which is not focused by the receiving optics, from reaching the photodetector **16**. (Hamamatsu Corporation S1723-04).

Although the use of single high F number parabola to image point sources is well known in telescopes, it is also well known that a low F number parabola, while efficient at collecting light, greatly distorts the image of light sources larger than single points. It was surprisingly discovered that two parabola of the same focal length, when placed with their verticies away from each other, open ends facing each other, create canceling distortions. In this configuration, even realistically sized sources of light are accurately imaged. The efficient light gathering ability of this configuration is applicable to light scatter, fluorescence, raman spectroscopy, plasma clot detection and other optical measurements and imaging. As this is a reflective optical system, it works well with a large range of light wavelengths. When viewing a light source through the plane surface of a cuvette, distortion of the image occurs due to refraction at the cuvette air interface. Part of this distortion can be canceled by tilting the parabolas but it was surprisingly discovered that a segment of an elipse chosen to have one of its vertex to focus lengths equal to the parabola it replaces cancels substantially more of the distortion caused by the cuvette, rendering an image spot 1/3 as large and 9 times as intense, as determined by computer analysis. An eliptical mirror of this type is easily fabricated by machining on a lathe with equipment similar to that used to fabricate the parabola it replaced. Thus, using a properly shaped and positioned ellipsoidal reflector near the cuvette corrects distortion caused by the refraction of light at the flat surface of the cuvette.

An optical encoder (Sumtak Model LHF-050-2000, not shown) was attached to the rotating optical assembly. The output of this encoder was used to provide rotational information to an IBM PCAT and digital data acquisition system. As the laser/detector optics assembly 1 passes under each cuvette the digital data acquisition system/computer digitized and stored the average of approximately 100 signal readings of the

amplified output of the detector 16, taken as the internal reflection surface 12 is scanned. Thus the average of approximately 100 readings obtained from each cuvette was stored with each rotation (one revolution approximately every 2 seconds). The readings were taken only from the center third of the cuvette to avoid errors arising from the inside radius of the cuvette well or the cuvette side walls. The computer also stored a reading proportional to the laser output, from another detector, and readings of a low scattering region (laser impinging on black anodized aluminum cuvette holder ring 19) and high scattering region (teflon block mounted in place of cuvette). These readings were used to compensate for variations in laser intensity and for detector drift. When the computer monitored the cuvettes for 10 minutes it generated a 5th order least squares approximation equation to the scatter signal vs. time data for each cuvette, subtracted the signal at time = 0 seconds from the curve and integrated this equation vs. time for each cuvette. This integral was then correlated with analyte concentration.

EXAMPLE 1

Hepatitis Virus Surface Antigen Test

Polycarbonate cuvettes, as depicted in Figure 3, were coated with mouse monoclonal anti-hepatitis surface antigen antibodies by incubating 200 microliters of a 100 microgram per ml solution of antibody in 0.01 M phosphate buffered saline, pH 7.4, overnight at room temperature followed by three aspirate fill steps with 300 microliters of 0.05 M Hepes/Tris, pH 8.3 buffer. The cuvettes were then overcoated with 300 microliters of 0.05 M Hepes/Tris, pH 8.3, buffer containing 1% bovine serum albumin for 60 minutes at room temperature, washed twice with 300 microliters of overcoating solution, incubated 15 minutes at room temperature with 300 microliters of 3% trehalose in 0.05 M Hepes/Tris, pH 8.3 buffer, aspirated, dried in room air and stored at room temperature in a desicator below 20% relative humidity. The cuvettes were then mounted in an instrument similar to that depicted in Figure 2 with the modification that the cuvette was rotated 180° about the illuminated surface 12 and the laser entered the cuvette while propagating away from the axis of rotation, still following the path inside the cuvette depicted in Figure 3.

Seventy-two microliters of standard, prepared by adding the appropriate amount of hepatitis surface antigen from (Merck) to a negative serum pool, was dispensed into cuvettes by an automated pipetter and allowed to incubate in the enclosed 37°C instrument for five minutes. The pipetter then dispensed 54 microliters of buffer (containing 2.0 M potassium chloride, 2% bovine serum albumin, 50 micrograms per ml of normal mouse IgG and 0.05% sodium azide dissolved in 0.05 M sodium barbital buffer at pH 8.5) and 180 microliters of 105 nm diameter 0.1% monoclonal anti-hepatitis surface antigen coated gold colloid suspension (in ten mM Hepes buffer, pH 7.0, containing 0.05% sodium azide, 300 mM mannitol, and 0.5% bovine serum albumin) at a rate sufficient to mix the fluids in the cuvette. The light scattered by each cuvette was then recorded for the next ten minutes. The time integral of the fifth order linear regression curve fit of the light scatter vs. time data was reported for each cuvette. The average of the signal from five of the six zero standards (one was 14 standard deviations from the mean of the other five) and the average of the duplicate standards correlated proportionately with the hepatitis surface antigen present, as can be seen from the following data:

HEPATITIS SURFACE ANTIGEN CONCENTRATION	MEAN SIGNAL
0	1.7358
0.1 ng/ml	2.2376
0.2 ng/ml	2.9421
0.4 ng/ml	3.99235
0.6 ng/ml	5.0442
0.8 ng/ml	6.72185
1.0 ng/ml	7.0187
1.5 ng/ml	9.31175
2.0 ng/ml	10.7365
2.5 ng/ml	14.0444
5.0 ng/ml	24.9279
10.0 ng/ml	47.4585

EXAMPLE 2

Anti-Hepatitis Core Antigen Human Antibody Test

Polycarbonate cuvettes, as depicted in Figure 3 were coated as in Example 1 (Second Set of Experiments), with recombinant hepatitis core antigen using a 5 microgram per ml coating solution. The cuvettes were then dried, stored and mounted in the instrument (Figure 2), which was enclosed and equipped with 37° air circulation. Seventy-two microliters of the appropriate sample or control were added to separate cuvettes by an automated pipetter and allowed to incubate for 5 minutes, after which time 54 microliters of assay buffer (consisting of 1% bovine serum albumin and 1 M NaCl dissolved in pH 7.4 phosphate buffered saline) and 180 microliters of a 0.1% suspension of 105 nanometer diameter mouse monoclonal anti-human IgG coated colloidal gold suspension was dispensed with sufficient velocity to mix the contents of the cuvette. The light scattered by the cuvette was then recorded for the next 10 minutes. The time integral of the fifth order linear regression curve fit of the scattered light vs time data was reported for each cuvette as signal. The mean signal of replicates of each serum sample correlated with the presence of anti-hepatitis core antigen employing a cuttoff of 3 standard deviations above the mean of the negative control, as shown in the following data:

SAMPLE TYPE	# OF REPLICATES	MEAN	TEST OUTCOME
Negative control	4	0.9524	S.D.=0.51
Positive control	4	60.2099	S.D.=6.5
Negative sample 1	2	2.1671	-
Positive sample 1	2	10.483	+
Positive sample 2	2	41.058	+
Positive sample 3	2	33.494	+
Positive sample 4	2	2.6043	+
Positive sample 5	2	74.2235	+

EXAMPLE 3

Plasma Clot Detection

An apparatus similar to that described in Example 1 and Figure 2 was employed to determine the clotting time (PT or Prothrombin time) of plasma samples. (This apparatus employed the three one-quarter wave retardation plates, as described above, to maintain polarization of the scanned laser beam and also employed two paraboloidal mirrors to collect light scattered by the clotting samples for quantitation.) Rectangular cuvettes (single cuvettes broken from a Roche Cobas cuvette ring) were mounted in the apparatus in place of the molded cuvette shown in Figures 2 and 3. Laser light impinging on the vertical surface of this cuvette was refracted at the surface of the cuvette below the critical angle and thus propagated through the sample in the cuvette, exiting on the opposite side from the illumination. Light scattered, by the proteins in solution in the sample, in a direction substantially toward the illuminations was gathered by the paraboloidal mirror nearest the cuvette (13, Figure 2) and concentrated by the second parabola (14, Figure 2) through the apperture (15, Figure 2) on the detector (16, Figure 2). Average readings of each scan of the cuvette were reported as in the previous Examples 1 and 2 as the optics rotated once every 1.1 second over the 120 second reading period. As the plasma clotted, the fibrin strands formed scattered light much more efficiently than fibrinogen monomer and thus the scattered light tracked the progression of clot formation with the point of inflection of the plot considered the clotting time. All reagents, cuvettes and the apparatus were maintained at 37 degrees C. The test was performed by pipetting 200 microliters of Ortho Brain Thromboplastin (OBT), lot # OBT 996 into the cuvette followed by 100 microliters of sample (either Ortho Plasma Coagulation control level I lot # 1PC 284 or level III lot # 3PC 748). The cuvette was mixed by rapidly aspirating and dispensing with the sample pipette. The instrument readings were then started. Figure 10 shows the data obtained from the clotting of a level I control with a point of inflection clotting time of 10 seconds. Figure 11 shows the data obtained from the clotting of a level III control with a point of inflection clotting time of 23 seconds. These times are in good agreement with data collected on an Ortho Diagnostics Koagulab 32-S coagulation instrument with the same reagents.

Third Set of Experiments

EXAMPLE 1

Test for Digoxin in Whole Blood Sample Using Scatter by Colloidal Gold of Light Incident at Non-Total Internal Reflectance Angles

The apparatus used in this Example is shown in Figures 5 and 6. Tests were performed on an acrylic cartridge **26** (Arden Medical systems) with a channel for the sample to flow through. The surface of the channel was coated with polyclonal goat-anti-digoxin antibody (Atlantic Antibody) by standard passive adsorption methods. Light **27** from a He-Ne laser **28** illuminated a portion of the coated surface **29** at an angle almost normal to the surface. At this angle of illumination, the light propagated into aqueous samples rather than producing an evanescent wave in the sample. Light scattered from the coated surface **29** was collected by a lens **30** and focussed onto an optical fiber **31**, which guided the light to a photomultiplier tube **32** (PMT). The output voltage of the PMT was digitized and recorded by a computer **33**. The detection system (lens and fiber) was carefully positioned and oriented so as to collect only light scattered from the coated surface **29** which propagated through the acrylic at angles between the plane of the surface and the critical angle.

Colloidal gold was prepared as described above (diameter approximately 40 nm.) and was coated with digoxin/bovine IgG. Whole blood was spiked with various amounts of digoxin. Samples containing equal mixtures of spiked whole blood and digoxin-colloidal gold were pipetted into the cartridge and the resulting light scatter was monitored over time.

Figure 7 shows typical results. In curve A, the sample contained no free digoxin, and the light scatter signal increased continuously during the seven minutes shown, as the digoxin-colloidal gold bound to the surface. In curve B, the whole blood sample contained approximately 4.5 µg/ml of digoxin which competes with the digoxin-colloidal gold for sites on the coated surface, and as a result the scatter signal leveled off after a few minutes. (The curves have been offset so that they start at the same level for comparison.).

In this example, the sample was below the coated surface. When the sample is above the coated surface, the settling of red blood cells causes large spurious scatter signals. For whole blood assays, applicants have found that it is preferable to orient the coated surface such that gravity pulls the red blood cells away from the surface, or at least does not pull them towards the surface.

EXAMPLE 2

Observation of Immunological Binding of Colloidal Gold with LED as Light Source

The apparatus used in this Example is shown in Figure 8. The tests were performed on the same acrylic cartridge **34** as in Example 1 (Third Set of Experiments). The light source **35** was a red light-emitting diode (LED, Stanley ER-300, 300 millicandela, 660 nm. peak wavelength, 30 nm. half-width). Light from the LED was focused on the coated surface **36** by a 10X microscope objective. Scatter from the surface was focused by a lens **37** on an aperture **38** and then to a PMT **39** with the same optical alignment conditions described in Example 1 (Third Set of Experiments). The LED output was electronically modulated at 1 KHz and the PMT output was demodulated at the same frequency by a lock-in amplifier **40** (PAR 128A) to improve signal-to-noise performance of the apparatus.

The reagent used in this Example was 40 nm. colloidal gold coated with goat-anti-mouse antibodies (Janssen "AuroProbe"). This reagent was pipetted into cartridges coated with Mouse IgG or Bovine Serum Albumin (BSA).

Figure 9 shows typical results. Curve C shows a substantial increase in scatter signal as the GAM-gold binds to a cartridge coated with MIgG. Curve D shows little response when the cartridge was coated with BSA.

Claims

1. An apparatus for scanning a plane polarized light source which comprises:
first polarization means for converting said plane polarized light to circularly polarized light;
means for directing said circularly polarized light received from said first polarization means; and
second polarization means for reconverting said directed circularly polarized light into plane polarized light so that the plane of polarization of said light will be dependent only upon the orientation of said means for reconverting said circularly polarized light into plane polarized light.

2. The apparatus of claim wherein said first and second polarization means comprise quarter-wave retardation plates.

3. The apparatus of claim or claim 2 wherein said directing means comprises a rotatable mirror.

4. A method of scanning a plane polarized light source comprising:
converting plane polarized light to circularly polarized light;
directing said circularly converted light; and
reconverting said directed circularly polarized light into plane polarized light so that the plane of polarization of said light will be dependent only upon the orientation of said means for reconverting said circularly polarized light into plane polarized light.

5. An apparatus for detection of light comprising:
first paraboloidal reflecting means;
second paraboloidal reflecting means whose axis is nearly coincident with and nearly symmetrical to said first reflecting means.

6. A method for detecting light comprising:
reflecting light from first paraboloidal reflecting means to second paraboloidal reflecting means whose axis is nearly coincident with and nearly symmetrical to said first reflecting means.

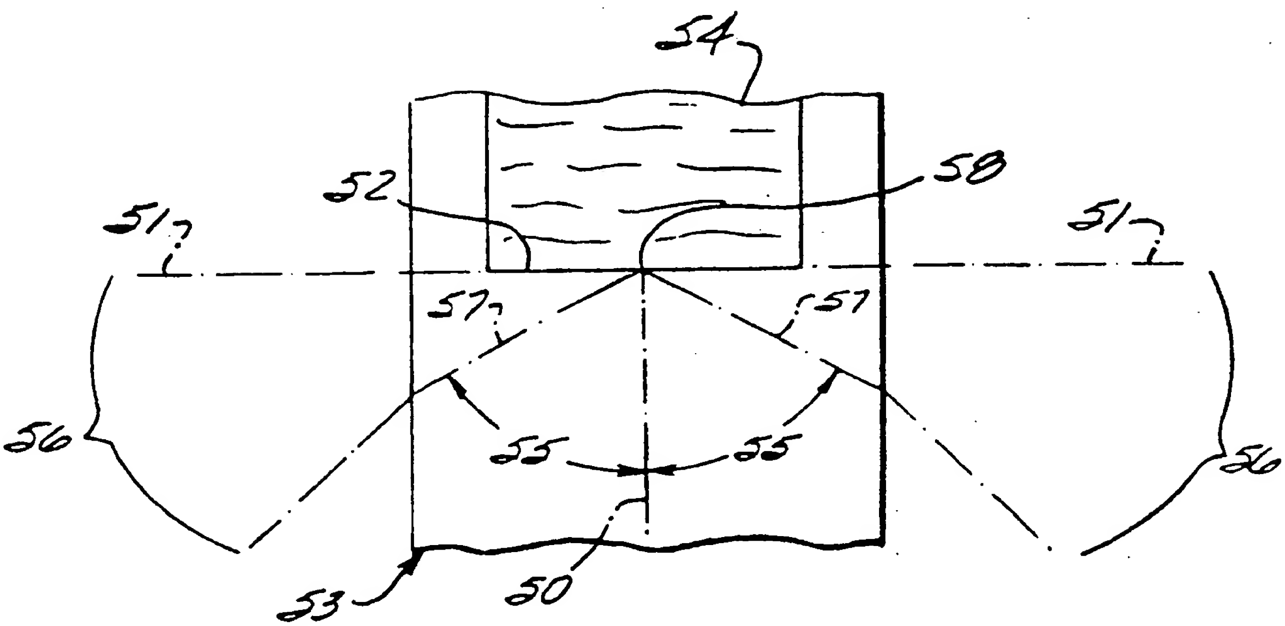
7. An apparatus for detection of light comprising:
first paraboloidal reflecting means;
second ellipsoidal reflecting means having its axis nearly coincident with and nearly symmetrical to said first paraboloidal reflecting means and its vertex to nearest focus distance substantially equal to the vertex to focus distance of said first paraboloidal reflecting means.

8. A method for detecting light comprising:
reflecting light from first ellipsoidal reflecting means to second paraboloidal reflecting means.

9. A light trap for dissipating the specular reflection of light occurring when substantially collimated light leaves a material with a refractive index greater than its surroundings comprising two planar surfaces of said material aligned relative to each other so that said substantially collimated light is multiply specularly reflected until said collimated light is substantially dissipated from said material.

10. A method for dissipating specular reflection occurring when substantially collimated light leaves a material with a refractive index greater than its surroundings comprising:
aligning two planar surfaces, preferably within 10 degrees of parallel, so that said substantially collimated light is multiply specularly reflected until said collimated light is substantially dissipated.

FIG-1



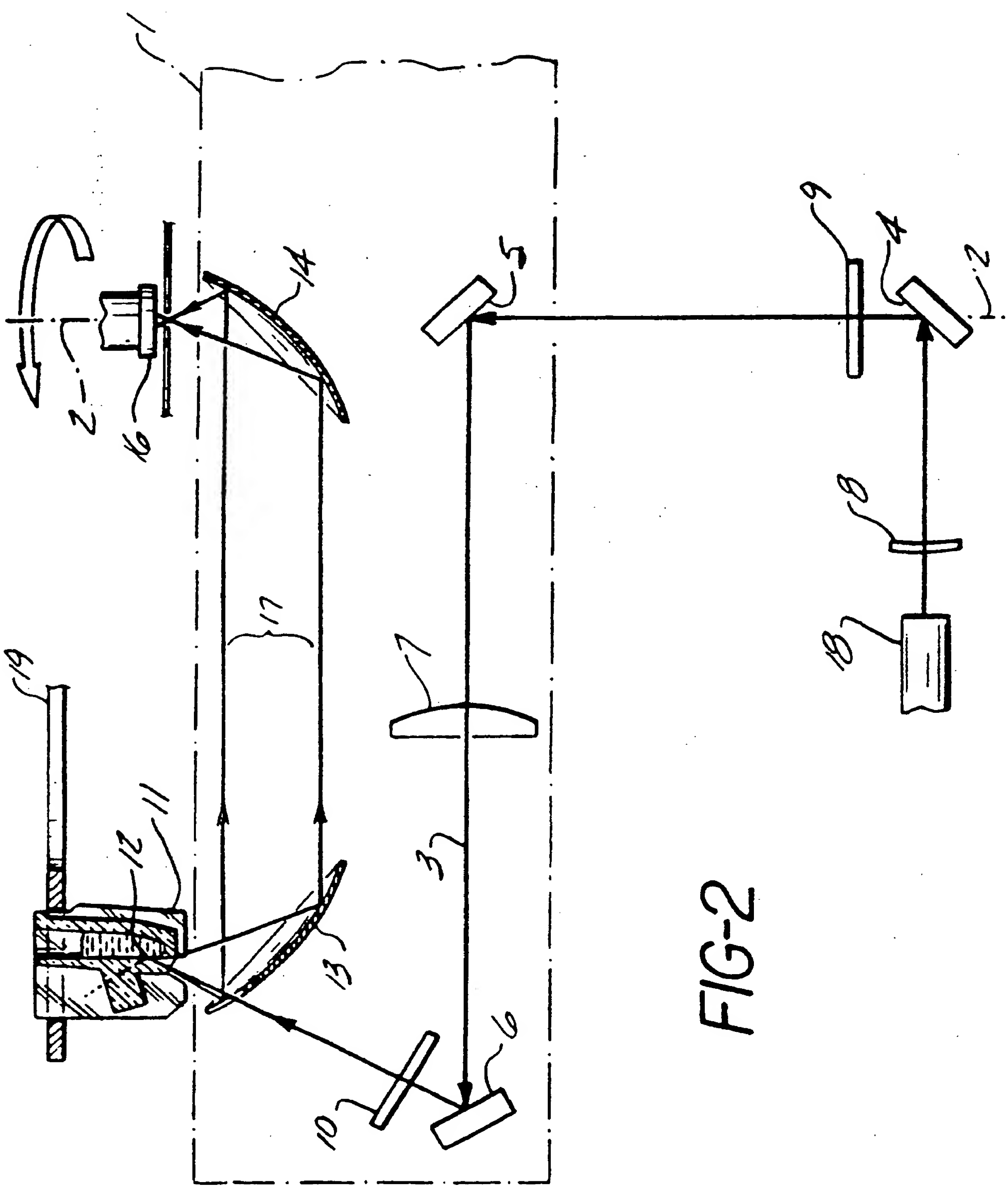


FIG-2

FIG-3

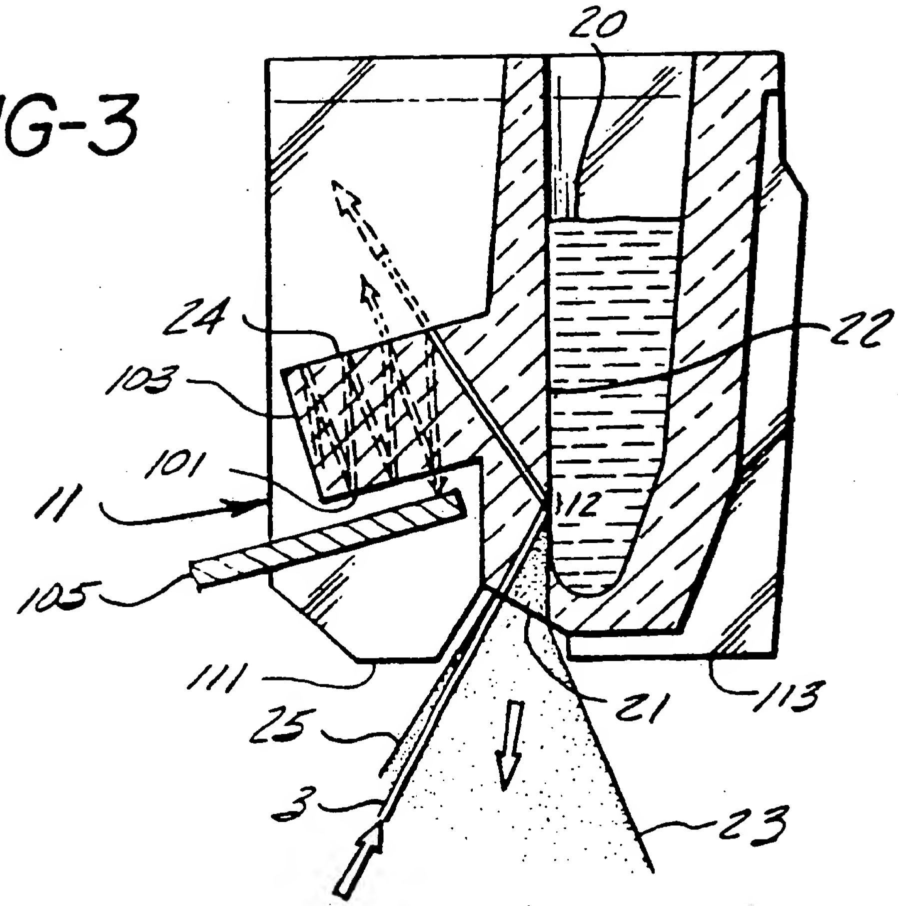
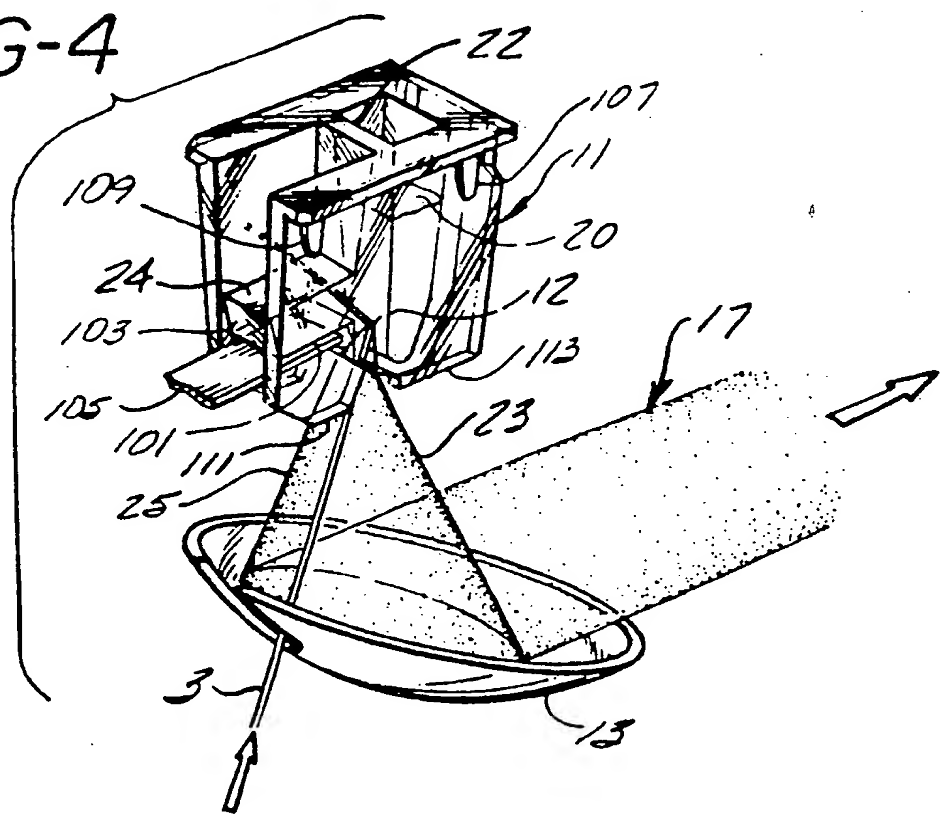

20
24
103
101
11
105
111
25
3
22
12
21
113
23

FIG-4


22
107
11
109
20
24
12
103
113
105
17
23
101
111
25
3
13

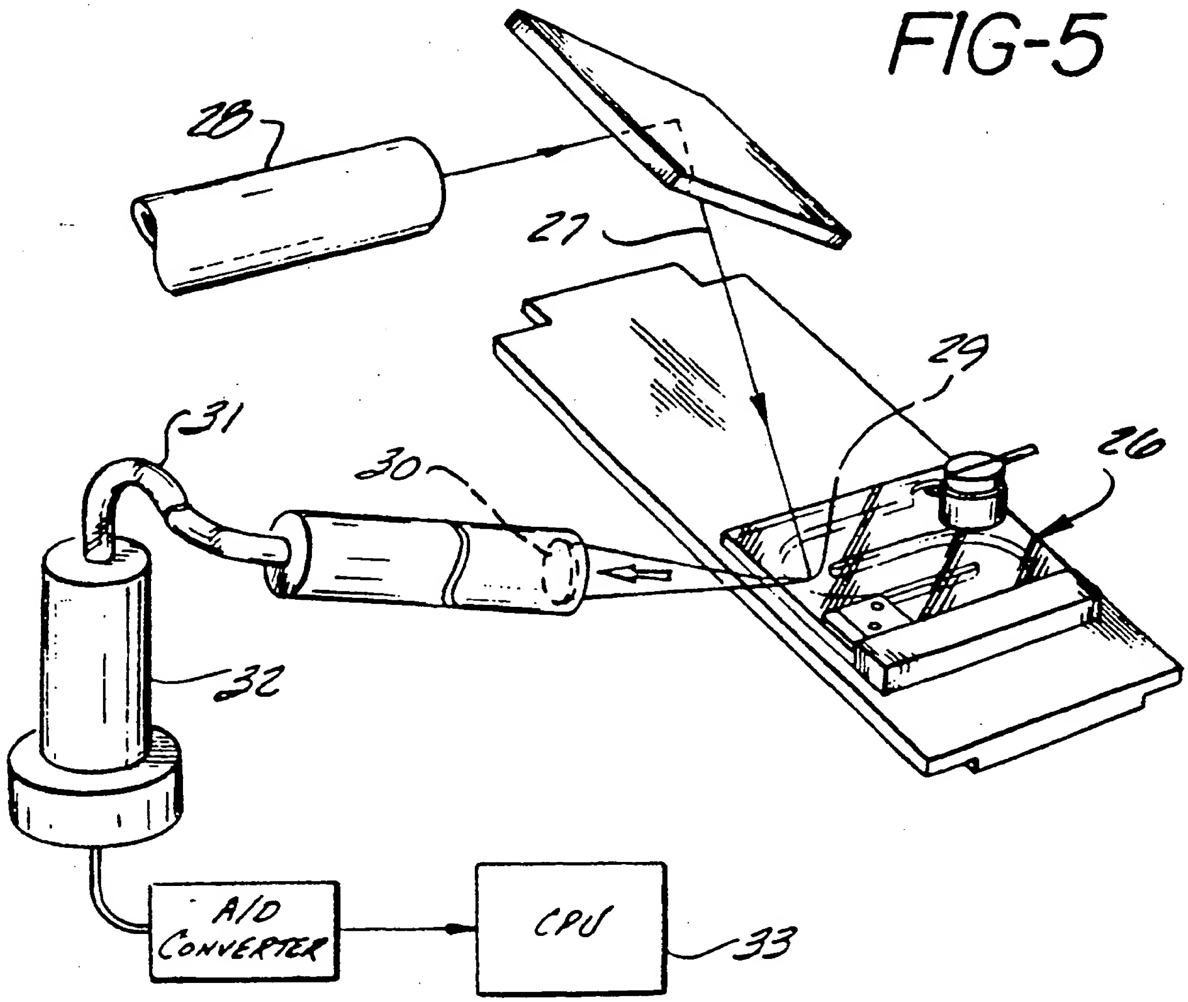


FIG-5

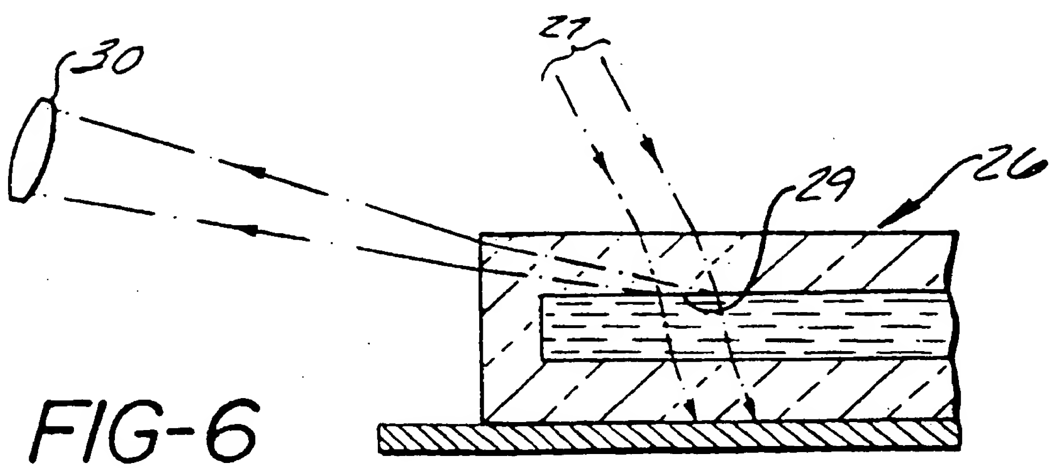


FIG-6

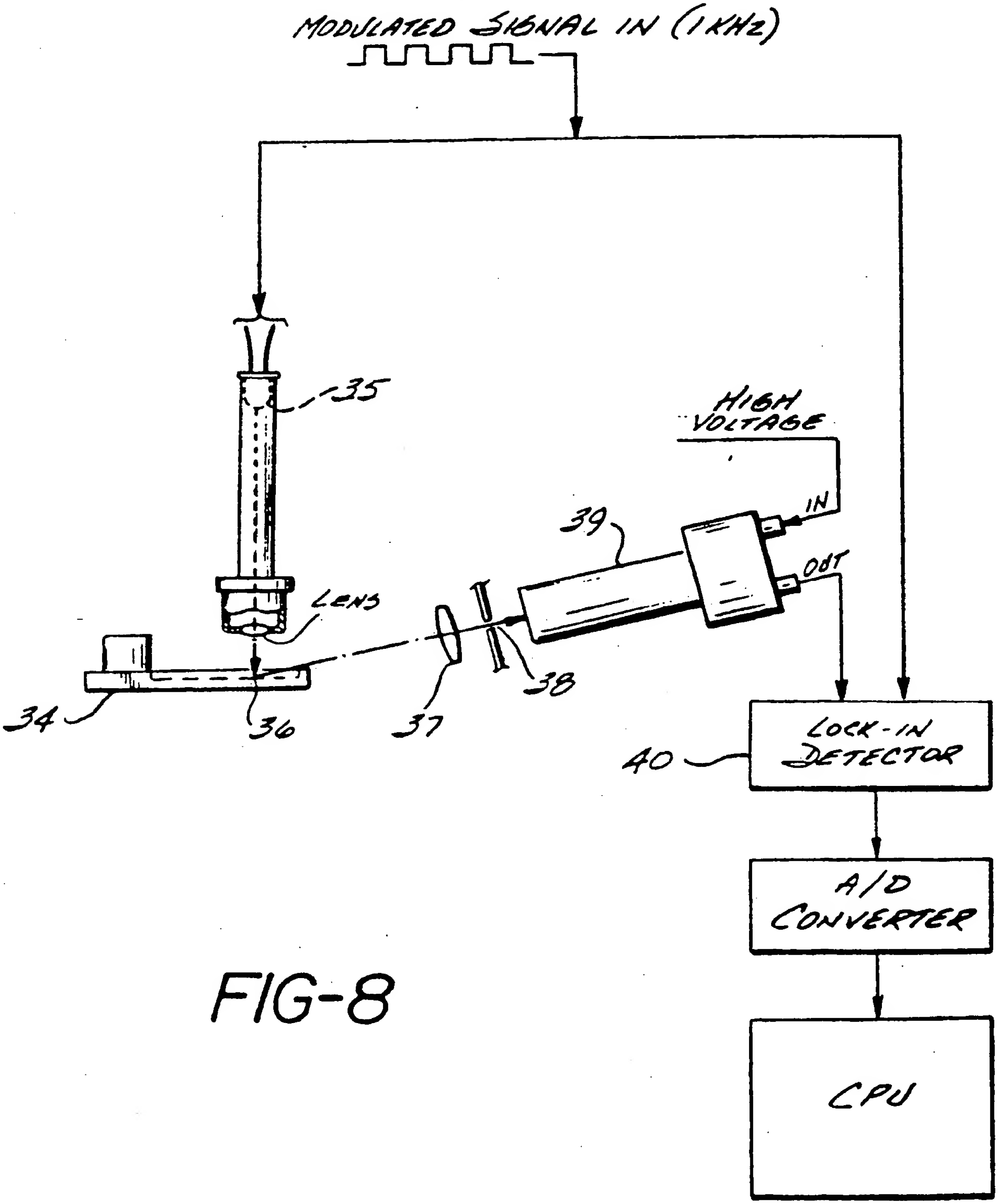

MODULATED SIGNAL IN (1 KHz)
HIGH VOLTAGE
IN
OUT
35
39
LENS
34
36
37
38
40
LOCK-IN DETECTOR
A/D CONVERTER
CPU

FIG-8

FIG-7

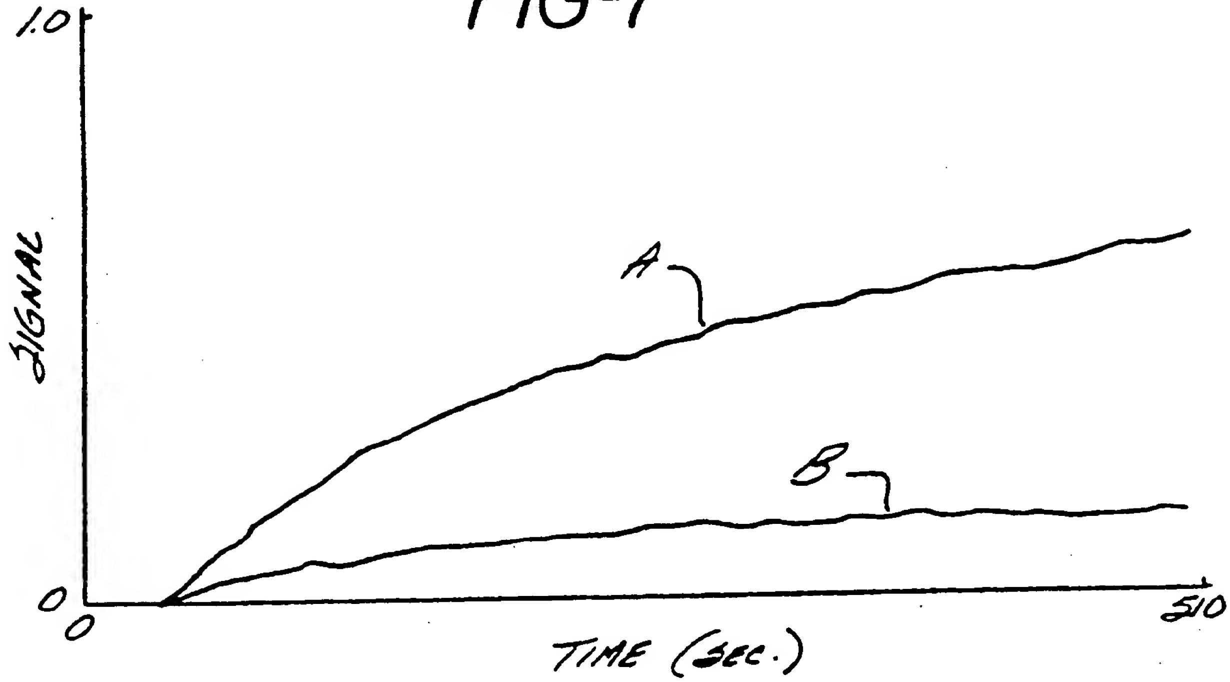


FIG-9

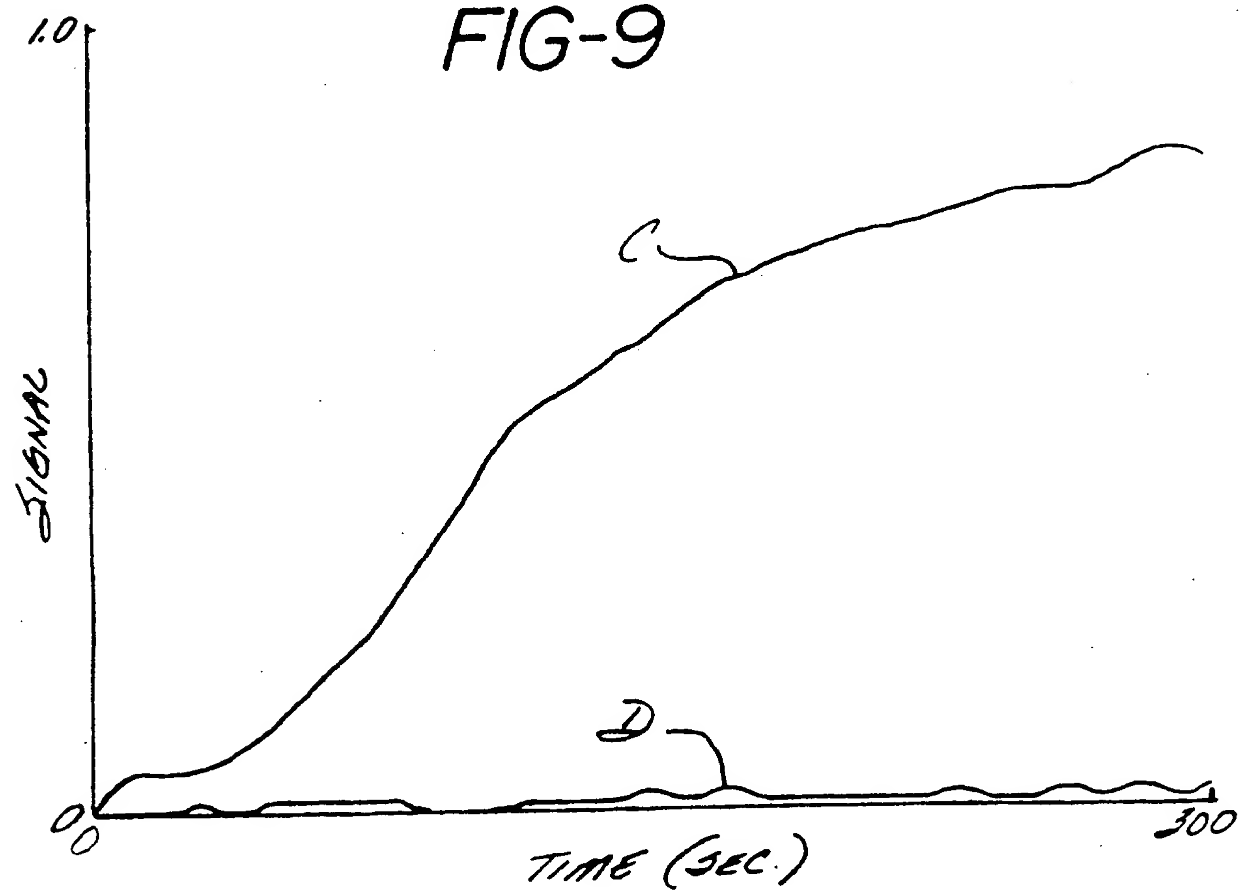


FIG-10

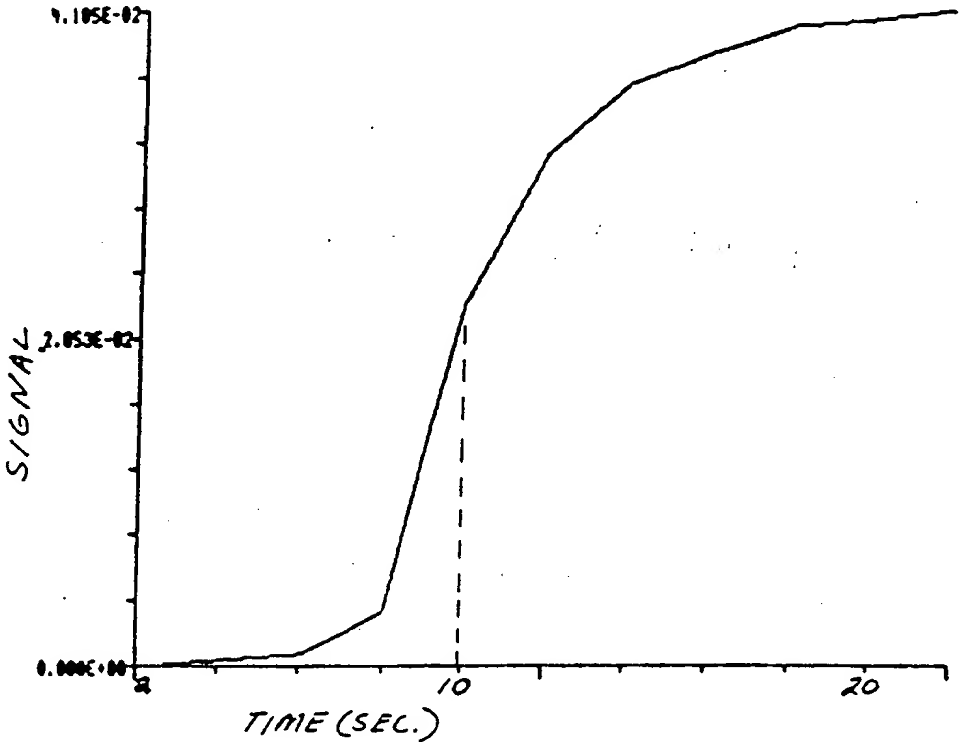
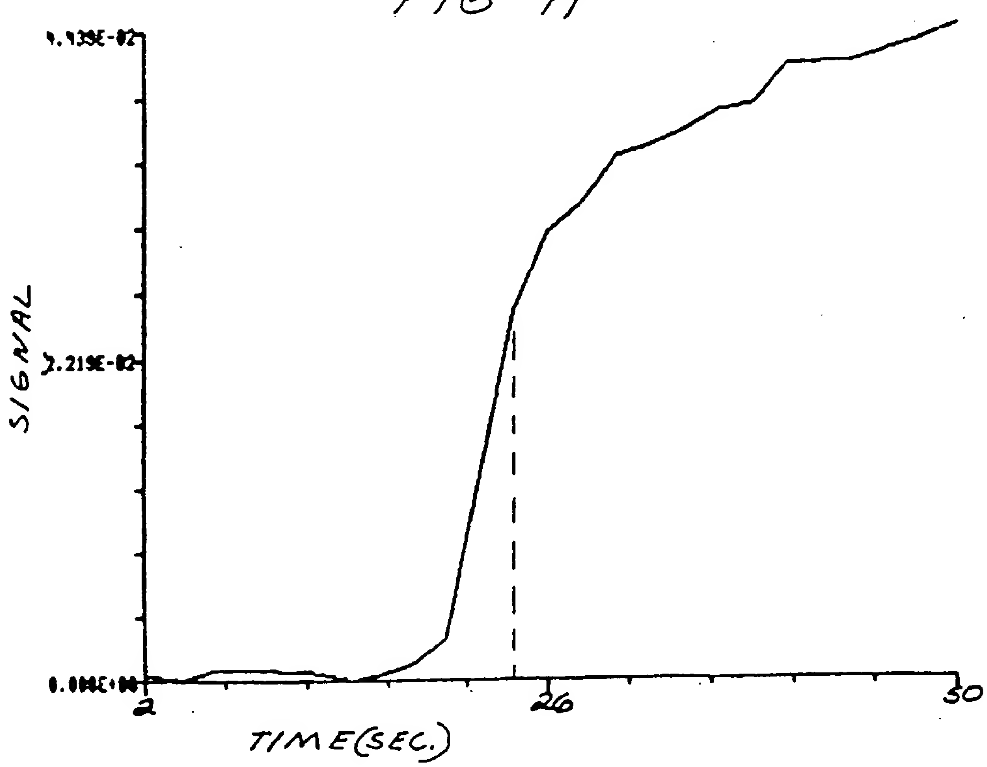

4.105E-02
2.053E-02
0.000E+00
SIGNAL
2
10
20
TIME (SEC.)

FIG-11


4.435E-02
2.219E-02
0.000E+00
SIGNAL
2
26
30
TIME(SEC.)